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EGG AND LARVAL DEVELOPMENT OF LABORATORY-REARED NASSAU GROUPER, *EPINEPHELUS STRIATUS* (PISCES, SERRANIDAE)

Allyn B. Powell and John W. Tucker, Jr.

ABSTRACT

Egg and larval development of the Nassau grouper, *Epinephelus striatus,* is described from laboratory-reared specimens. Egg diameters averaged 0.92 mm (0.86–0.97 mm), and those of the single oil globule averaged 0.24 mm (0.20–0.26 mm). No pigmentation was discernible on embryos. Newly hatched larvae measured 1.7–1.8 mm notochord length, and were inconspicuously pigmented. A characteristic pigment pattern that persists during larval development first appeared on late yolk-sac larvae—a mass of pigment on the ventral midline and lateral surface of the caudal peduncle, and on the dorsal and lateral surface of the gut. The enlarged, serrated, second first-dorsal-fin and pelvic spines that are characteristic of epinephelin larvae formed very early in the preflexion stage, but spinelets were not well developed until postflexion. The adult complement of dorsal-fin and anal-fin spines and rays was first observed on specimens approximately 6.8 mm standard length (SL) based on pterygiophores to obtain counts. But the appearance of bony stays, which signified completion of the dorsal and anal fins was not complete until 7.4 mm and 7.0 mm SL, respectively. Separation of preflexion and flexion *E. striatus* from all other epinephelin groupers does not appear possible until comparative studies of pigment patterns, second-dorsal-fin pterygiophore patterns, ceratobranchial gill raker counts, and spinelet development can be done. *E. striatus* postflexion larvae longer than 7.4 mm SL can be separated from all other epinephelin larvae except *E. adscensionis* on the basis of dorsal and anal fin ray counts, spinelet configuration, second first-dorsal-fin spine length relative to standard length, and capture location.

The Nassau grouper, *Epinephelus striatus,* ranges from Brazil to North Carolina (Böhlke and Chaplin, 1968). It is primarily an insular species and is most common in the West Indies, Bahamas, southern Gulf of Mexico, Colombian Caribbean, and Venezuelan Archipelago Los Roques (Jory and Iversen,1989). *Epinephelus striatus* may spawn from January through April near Jamaica (Thompson and Munro, 1983), May through August near Bermuda (Smith, 1971), and December through March near Grand Cayman.[1] A brief description of its spawning behavior in captivity, along with notes on early development, which are not useful for larval identification, was given by Manday and Fernandez (1966).

This paper describes *E. striatus* from the egg to late larval stage from laboratory spawned and reared material.

METHODS

Adult *E. striatus* used for spawning were collected during 1987, 1988, 1989 and 1990 near Grand Cayman. Ripe females were induced to ovulate with injections of human chorionic gonadotropin. Eggs were manually stripped and fertilized with milt from naturally running ripe males. Larvae were reared in unrecirculated natural seawater passed through a 5-μm filter at temperatures from 23 to 30°C, and salinities from 31 to 38‰.

Two developmental series of larvae were used. The first series was used to describe pigment patterns, and illustrate larval stages. Specimens in the second series were stained with alcian blue and alizarin

[1] Tucker, J. W., Jr. and P. G. Bush. Spawning locations and times of Nassau grouper (*Epinephelus striatus*) near Grand Cayman. Unpubl. manuscr. Harbor Branch Oceanographic Institution, Fort Pierce, Florida 34946.

red and were cleared with trypsin according to Dingerkus and Uhler (1977), as modified by Potthoff (1984). These specimens were used to describe meristic characters and to obtain measurement of the second first-dorsal fin, first pelvic fin, and preopercular spines. The adult complement of dorsal, anal and pectoral fin-ray counts was taken from Johnson and Keener (1984), branchiostegal and gill raker counts from Smith (1971) and caudal fin-ray counts from Miller and Jorgenson (1973). The larval period was separated into yolk-sac, preflexion, flexion, and postflexion stages—the latter three stages associated with the development of the caudal fin before, during, and after the upward flexion of the notochord tip and development of principal caudal rays. Measurements from larvae preserved in 5% buffered Formalin are as follows:

Body Length.—In yolk-sac, preflexion, and flexion larvae, the horizontal distance from the tip of the snout to the tip of the notochord (notochord length = NL). In postflexion larvae (after the full complement of principal caudal rays are formed), from the tip of the snout to the base of the hypural bones (standard length = SL).

Second first-dorsal fin spine and first pelvic fin spine lengths were from their structural base to their tip on a flat plane. Measurements were made on cleared and stained specimens.

Preopercular Spine Length.—From the posterior edge of the exterior shelf of the preopercular (Potthoff et al., 1984) to the tip of the serrate spine at the angle of the preopercular.

RESULTS

Eggs and Recently Hatched Larvae.—*Epinephelus striatus* eggs were spherical and averaged 0.92 mm in diameter (0.86–0.97 mm, N = 100). One oil globule was present and it averaged 0.24 mm in diameter (0.20–0.26 mm, N = 52). The yolk was homogeneous, the chorion smooth, and the perivitelline space narrow. No pigment was discernible on embryos from middle stage (blastopore closure until the tail twists out of the plane of the embryonic axis) or late stage (from tail twisting to hatching) eggs. Eggs floated in water of more than 32‰ salinity. They hatched within 27–29 h after fertilization at 25°C, and 23–25 h at 28°C.

Newly hatched larvae measured 1.8 mm NL and were slightly curved around the yolk sac. The oil globule, in the majority of specimens, was located in the ventroposterior area of the yolk sac. Rarely was it located anteriorly.

Fin Development.—Fins began development in the following sequence: pelvic, first dorsal, caudal, pectoral, anal and second dorsal (Table 1). The adult complement of spines and soft rays was attained in the following sequence: principal caudal rays (9 upper + 8 lower), first dorsal fin (X1) and anal fin (III, 8), second dorsal-fin rays (16–17) and pelvic-fin spines and soft rays (I, 5), pectoral-fin rays (18–19), and secondary caudal rays (10 upper and 9 lower) (Table 1).

The development of the caudal fin, indicated by a thickening of tissue on the ventral side of the notochord, began at 5.0 mm NL. Caudal-fin rays (2 upper + 2 lower) were first observed at 5.4 mm NL (Table 1). The rays began to form at the middle of the fin and developed dorsally and ventrally simultaneously. The adult complement of principal caudal-fin rays was observed at 6.0 mm SL (Table 1). The adult complement of secondary caudal rays appeared to be attained between 9.4 and 12.7 mm SL (Table 1).

The second dorsal spine was the first spine to form on the first dorsal fin. A thickened area of tissue above the notochord on a 2.9 mm NL preflexion larva was the first indication of spine development (Fig. 1C). The first dorsal-spine was next to form (4.2 mm NL) followed by the third at 4.6 mm NL. Further, spine development proceeded posteriorly (Fig. 2). The adult complement of dorsal spines was attained during the postflexion stage at approximately 6.6 mm SL. Soft rays in the second dorsal fin did not begin to form until the postflexion stage, but then rays were added rapidly (Table 1, Fig. 2). Rays number 2 through 7 were formed at 6.0 mm SL, and rays number 1 through 12 at 6.8 mm SL. At 7.0 mm SL the adult complement of soft rays on the second dorsal fin was attained (Table

Table 1. Meristic data from cleared and stained laboratory-reared larval Nassau grouper, *Epinephelus striatus* (dashed lines separate preflexion, flexion, and postflexion stage larvae)

Body length (mm)	Days after hatching	Dorsal fin		Anal fin	Pelvic fin	Left pectoral	Principal* caudal rays	Secondary* caudal rays	Branchio-stegal rays	Gill rakers (left first arch)		
		Spines	Rays							Epibranchial	Cerato-branchial†	Hypobranchial
2.4	4	–	–	–	–	–	–	–	–	–	–	–
3.1	7	–	–	–	–	–	–	–	–	–	–	–
3.3	12	–	–	–	I	–	–	–	–	–	–	–
3.4	7	–	–	–	–	–	–	–	–	–	–	–
3.7	10	I	–	–	I	–	–	–	–	–	–	–
3.9	12	I	–	–	I	–	–	–	–	–	–	–
4.1	10	I	–	–	I	–	–	–	3	–	–	–
4.2	12	II	–	–	I	–	–	–	4	–	5	–
4.2	10	I	–	–	I,1	–	–	–	3	–	–	–
4.5	12	II	–	–	I	–	–	–	4	–	5	–
4.6	16	III	–	–	I,2	–	–	–	5	–	4	–
4.9	13	III	–	–	I,2	–	–	–	5	–	6	–
5.0	16	III	–	–	I,2	–	–	–	5	–	6	–
5.4	16	III	–	–	I,2	–	2+2	–	6	–	7	–
5.5	17	IV	–	–	I,3	–	5+4	–	7	–	9	–
5.5	17	III	–	–	I,2	–	1+2	–	5	–	9	–
5.7	17	IV	–	–	I,3	–	6+5	–	7	–	9	–
5.7	16	III	–	–	I,3	1	5+5	–	7	1	9	–
6.0	17	IV	–	–	I,4	–	6+6	–	7	–	10	–
6.0	25	IV	–	I,4	I,4	2	8+8	–	7	3	11	1
6.2	17	IV	–	–	I,3	3	6+6	–	7	–	11	–
6.5	17	IV	–	II,5	I,4	5	8+8	–	7	–	10	–
6.5	20	V	–	II,5	I,4	5	8+8	–	7	2	10	–
6.0	17	V	6	II,5	I,4	7	9+8	0+1	7	2	10	–
6.6	21	XI	9	III,6	I,3	4	9+8	0+1	7	3	11	–
6.8	25	XI	12	III,8	I,4	9	9+8	0+2	7	4	11	3
7.0	21	XI	17	III,8	I,5	9	9+8	0+1	7	3	11	1
8.5	26	XI	17	III,8	I,5	14	9+8	5+5	7	5	11	3
9.1	26	XI	17	III,8	I,5	15	9+8	7+7	7	6	11	3
9.4	30	XI	17	III,9	I,5	19	9+8	7+7	7	6	11	4
12.7	38	XI	16	III,9	I,5	19	9+8	11+11	7	8	12	4
13.2	40	XI	16	III,8	I,5	18	9+8	9+9	7	6	11	4

* Upper + lower rays.
† Includes gill raker at epi-ceratobranchial angle.

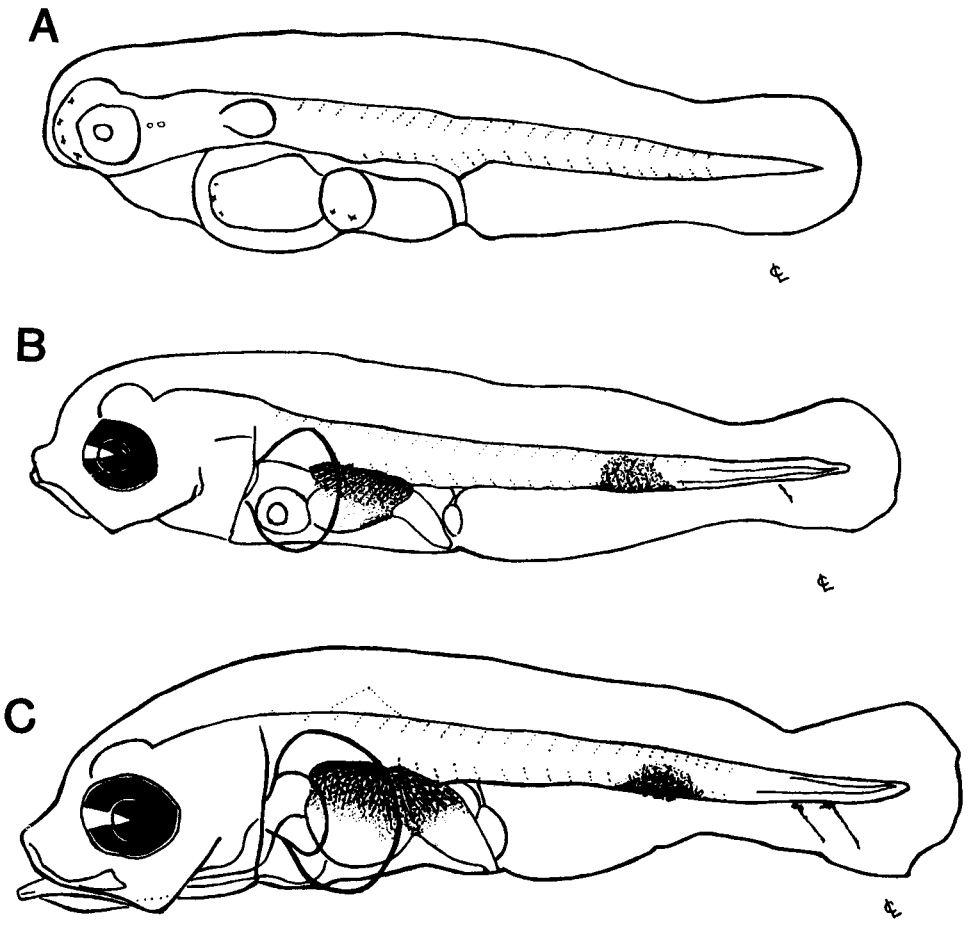

Figure 1. Developmental stages of laboratory-reared *Epinephelus striatus*: A, 2.5 mm NL early yolk-sac larva, 1 day old; B, 2.6 mm NL late yolk-sac larva, 3 days old; C, 2.9 mm NL preflexion larva, 5 days old.

1), but the bony stay that signifies the development of the posteriormost dorsal-fin soft ray was not present until 7.4 mm SL.

Anal-fin spines and soft rays initiated formation on flexion larvae at approximately 6.0 mm NL (Table 1). At this size we observed the second anal-fin spine and the first four soft rays. On late postflexion larvae (6.5 mm NL) the second and third anal-fin spine was observed along with the first five soft rays (Fig. 2). This condition remained during early postflexion (6.5 mm SL). By 6.6 mm SL the first anal-fin spine began to form (Fig. 2) and at 6.8 mm SL the adult complement of anal-fin spines and soft rays was attained (Table 1), but the bony stay that signifies the development of the posteriormost anal-fin soft ray was not present until 7.0 mm SL. In summary, the completion of the first and second dorsal fin, and anal fin along with their respective bony stays was observed at 7.4 mm SL.

The first spine of the pelvic fin (the first fin to begin formation) was first observed on a preflexion stage larva at 3.3 mm NL (Table 1). Soft ray development was first observed at 4.2 mm NL with further development of soft-rays occurring

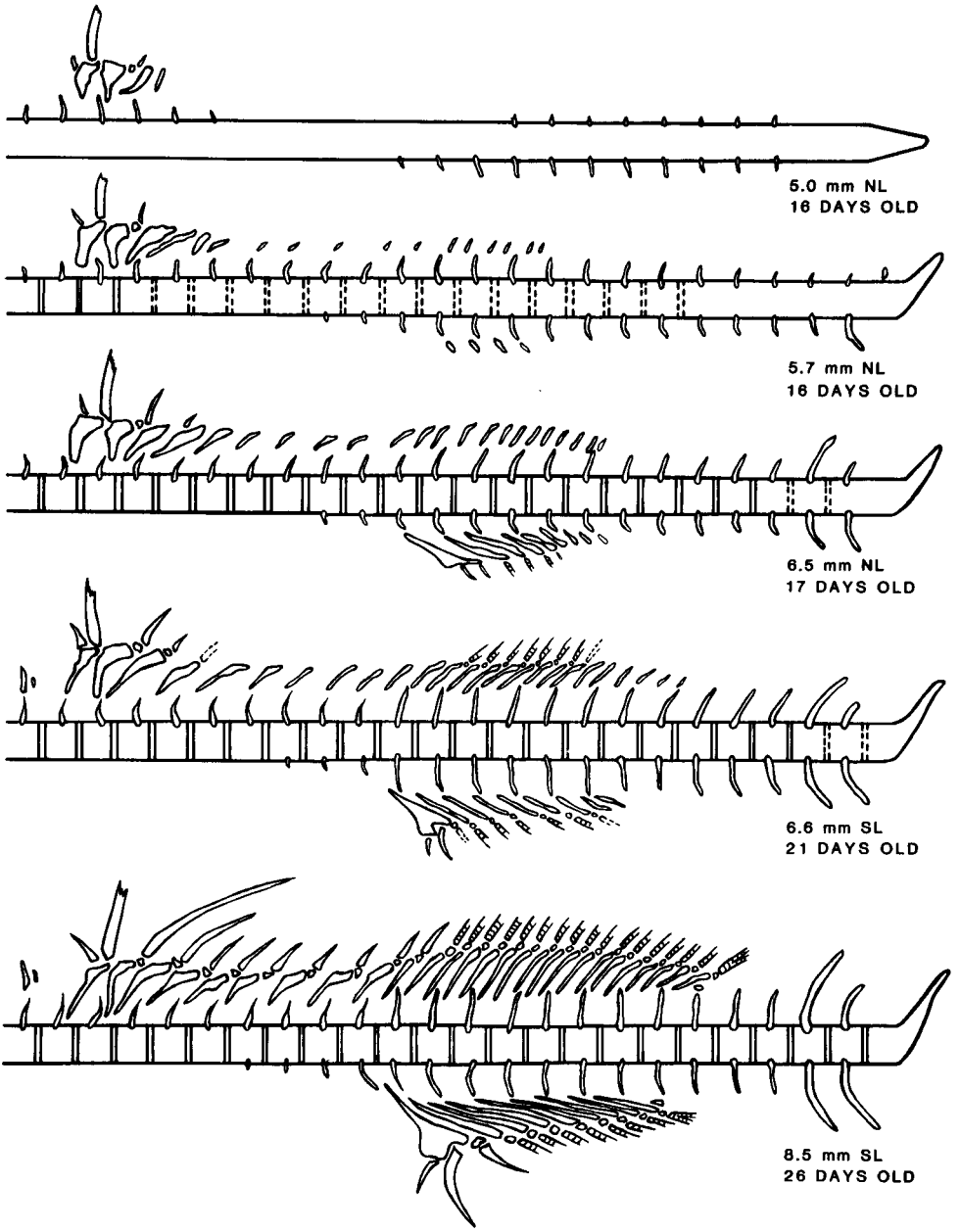

Figure 2. Schematic representation of the development of the vertebral column and dorsal and anal fins in laboratory-reared *Epinephelus striatus*.

relatively slowly. All specimens ≥7.0 mm SL had a completed pelvic fin Table 1).

The pectoral fin persisted as rayless blade throughout the preflexion and early flexion stages (Table 1). Rays begin to form at 5.7 mm SL at the dorsal position of the blade and then developed ventrally. All specimens ≥9.4 mm SL had a complete pectoral fin (Table 1).

Table 2. Frequencies of dorsal fin pterygiophores between neural spines in 12 *Epinephelus striatus* (6.0–30.0 mm SL). When a full complement of pterygiophores did not appear to be formed between neural spines (on a 6.0 mm-SL larva), counts were omitted. When the proximal end of the pterygiophore was in alignment with the distal end of the neural spine, the pterygiophore was not included in the counts.

Neural spine/centrum number	No. of pterygiophores between neural spines				Neural spine/centrum number	No. of pterygiophores between neural spines			
	0	1	2	3		0	1	2	3
2–3	—	12	—	—	11–12	—	—	12	—
3–4	—	—	12	—	12–13	—	—	12	—
4–5	—	12	—	—	13–14	—	—	12	—
5–6	—	12	—	—	14–15	—	—	8	4
6–7	—	12	—	—	15–16	—	—	9	2
7–8	—	12	—	—	16–17	—	1	10	—
8–9	—	12	—	—	17–18	—	—	5	6
9–10	—	12	—	—	18–19	2	6	1	1
10–11	—	9	3	—					

Dorsal and Anal Fin Supports.—Pterygiophore development first occurred at 3.7 mm NL. The first support to form appeared to be the second first-dorsal-fin pterygiophore that secondarily supports the elongated second first-dorsal-fin spine (see Kendall (1976) and Johnson (1983) for a detailed description of the predorsal bones and anterior dorsal pterygiophores in adult *Epinephelus* sp.). Next to form was the anteriormost pterygiophore that secondarily supports the first dorsal spine. First dorsal-fin pterygiophores then formed anterior to posterior, although on a 5.7 mm NL specimen a faint pterygiophore was observed that appeared to be the posteriormost pterygiophore serially supporting the last spine of the first dorsal fin (Fig. 2).

Pterygiophores supporting dorsal-fin soft rays were first observed between neural spines 12 and 14 at 5.7 mm NL. Pterygiophores then formed both anteriorly and posteriorly simultaneously, and at 6.0 mm NL the anteriormost pterygiophores supporting the second-dorsal-fin soft rays was formed. The adult complement of second dorsal-fin pterygiophores was attained at 6.8 mm SL during the postflexion stages (Fig. 2).

Anal fin pterygiophores were first observed at 5.7 mm NL and were forming in proximity to haemal spines 2–4 (Fig. 2). Anal fin pterygiophores formed rapidly. A 6.0 mm NL specimen had eight pterygiophores forming in proximity to haemal spines 1–5, while on another specimen of the same size we observed the first pterygiophore that supports the three anal-fin ray spines and eight pterygiophores that will support the full complement of anal-fin soft rays. All specimens $\geq$6.5 mm NL had the full complement of anal-fin pterygiophores (Fig. 2).

The arrangement of dorsal-fin pterygiophores relative to neural spines in conjunction with other characters could be of taxonomic value (Ahlstrom et al., 1976; Dunn, 1984). We examined late larval ($\geq$6.0 mm SL) *E. striata* to identify a dorsal-fin pterygiophore pattern. Our results are limited as we had relatively few specimens, and a comparative study between taxonomically similar species is necessary to establish the value of pterygiophore patterns.

The arrangement of predorsal bones, and the first three first-dorsal-fin pterygiophores in some adult serranid genera has been well studied (Kendall, 1976; 1984; Johnson, 1983). The arrangement of these elements (Fig. 2, Table 2) is useful in establishing relationships between subfamilies but has little taxonomic value for larval identification of epinephelin serranids.

The arrangement of pterygiophores in association with spines of the first dorsal fin, except for the condition between the 10th and 11th precaudal centrum, was similar for all specimens (Table 2, Fig. 2). Beginning with the 4th pterygiophore (which occurred between the 4th and 5th centrum and was serially associated with the 5th spine) a single pterygiophore occurred between precaudal centra (Fig. 2). The pterygiophore that was serially associated with the 11th spine always occurred between the 10th and 11th centrum. In most larvae this pterygiophore occurred solely between neural spines (Fig. 2), but in a few larvae a pterygiophore in serial association with the most anterior soft ray also occurred between the 10th and 11th centrum (Table 2). The arrangement of pterygiophores associated with first-dorsal-fin spines in *E. striatus* was similar to that of *E. guttatus* (Smith, 1971) and as most species of *Epinephelus* share the same first-dorsal-fin spine counts (Johnson and Keener, 1984) it is likely that most species would share similar pterygiophore arrangements.

The arrangement of pterygiophores in association with soft rays was more variable than that associated with spines, but a consistent pattern between certain neural spines was observed (Table 2). Still, the taxonomic value of soft-ray pterygiophore patterns can only be established when a comparative study of taxonomically similar species is undertaken.

Other Structures.—Centra were not fully differentiated until after postflexion (7.4 mm SL), but the full complement of precaudal (10) and caudal (14) vertebrae was determined from larvae by 5.4 mm NL by counting combinations of neural and haemal spines. The first caudal vertebrae was easily identified as its haemal spine was distinctly longer than the preceding parapophysis. The last haemal spine was easily identified as the parahypural was formed at this size.

Branchiostegal rays first appeared during the preflexion stage (4.1 mm NL) and attained the adult complement (7), which is shared by all *Epinephelus* and allied genera (Smith, 1971), during early flexion (5.7 mm NL) (Table 1).

The adult complement of total gill raker counts (22–26) was not attained by any larvae we examined; therefore, total counts would not be a useful taxonomic character. On the other hand, the full complement of ceratobranchial gill rakers appeared to be formed during the flexion stage (6.0 mm NL). Compared to *E. adscensionus*, which shares identical fin ray counts and spine morphology (Johnson and Keener, 1984), *E. striatus* has slightly less total gill raker counts. Based on Smith's (1971) frequency distributions ≥80% of *E. striatus* and *E. adscensionus* had 24–25 and 25–27 gill rakers, respectively. A comparative study of ceratobranchial gill rakers might be useful in separating epinephelin serranids.

The ratios of the second first-dorsal-fin spine and first pelvic spin lengths to body length, which may be good characters for distinguishing species (Johnson and Keener, 1984), exhibit considerable allometric growth during the larval stage (Fig. 3). The ratio of the second first-dorsal-fin spine length to body length was greatest (0.47) for larvae 7.0 mm SL. The ratio declined to 0.25 for the largest specimen examined (Fig. 3B). The ratio of first pelvic-spine length to body length was also greatest (0.32) for larvae 7.0 mm SL. The ratio declined to 0.20–0.30 for larvae approximately 8.5–13.5 mm SL. The pelvic spine appears to have two stanzas of growth—the first during the preflexion stage (≤5.0 mm NL) and the second during the flexion stage (>5.0 mm NL) (Fig. 3A). These apparent discrete growth stages may be more a result of measuring curved spines on a flat plane than allometric growth. Regardless, the stage of larvae as well as body length should be considered if pelvic spine length is to be used as a diagnostic character. The ratio of preopercular spine length to body length also increased with body

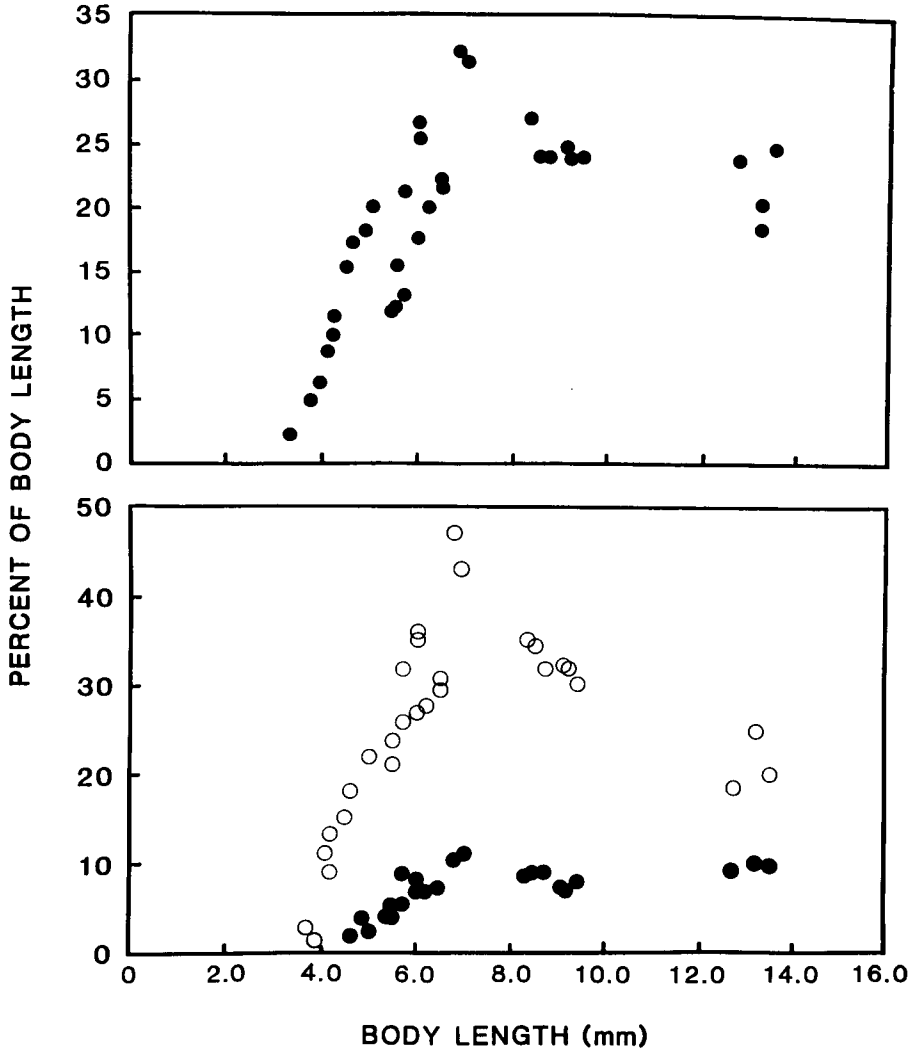

Figure 3. The proportion of *Epinephelus striatus* spine length relative to body length: upper, pelvic-spine length (solid circles); lower, preopercular-spine length (solid circles), second-spine length of the first dorsal fin (open circles).

length but the increase was considerably less than with the first pelvic and second first-dorsal-fin spines (Fig. 3B).

Serration patterns on spines, especially on the pelvic spine, are good diagnostic characters (Johnson and Keener, 1984). We observed the initial development of serrations on the pelvic spine of a 4.1-mm NL preflexion larva (cleared and stained material, 100× magnification). In that specimen five to six serrations (scallops in the membranes) were located at the base of the pelvic spine and none on the second first dorsal-fin spine. Larvae 4.6–5.0 mm NL had serrations on both the second first-dorsal-fin and pelvic spines extending from the bases to approximately 65–75% of the spine lengths. On these specimens the spinelets (stained alcian blue) were connected to themselves and the spine by a membrane. There were

no major characteristic changes until larvae reached a size of 6.0 mm NL, when the serrations on both the pelvic and second first-dorsal-fin spines extended fully to the distal end of the spines. Serrations on the dorsal spine appeared to be relatively more developed than on the pelvic spines. On young postflexion larvae (6.8 mm SL) the second first-dorsal-fin and pelvic spinelets were well developed at the base of the spines, but at the distal edge of the spine the membrane was scalloped (serrated), but spinelets had not yet formed. Hence, development of spinelets proceeded from the base to the distal end of the spine, with each spinelet growing from the edge of the membrane toward the spine.

Pigmentation.—Newly hatched larvae had several small, faint, dendritic melanophores on the snout. Dendritic melanophores also were present on the anterior surface of the yolk sac and on the oil globule (Fig. 1A). Yolk-sac larvae in which the mouth was beginning to develop had a characteristic pigment pattern that remained during larval development. Melanophores occurred in a distinct "inverted saddle" on the ventral midline and lateral surface of the caudal peduncle (Fig. 1B). The dorsal extension of the pigmented inverted saddle on the lateral surface varied among specimens. In some it almost encircled the notochord. Another persistent pigment mass was on the dorsal and lateral surface and, occasionally, the ventral surface of the gut (Fig. 1B, C). In almost all specimens examined this mass of pigment was most dense on the dorsal surface. In later yolk-sac larvae and early preflexion larvae (2.5 mm NL) pigment appeared on the fin-fold in the area where the caudal fin would begin to form (Fig. 1B, C). In later yolk-sac larvae (2.5 mm NL), this pigment might appear as one to a few faint punctate melanophores, with branches extending from the melanophores. On late yolk-sac/early preflexion larvae, this pigment might be present or absent and the number of punctate and branching melanophores is variable. Characteristic pigment on preflexion larvae (from 2.7–2.9 NL to flexion), was the inverted saddle on the caudal peduncle, mass of pigment on the gut, and pigment in the caudal fin-fold (Fig. 1C). Pigment on both the gut and caudal peduncle varied in intensity. Almost all specimens had pigment in the caudal fin-fold area at this stage of development, but this pigment was not observed during flexion.

Pigment patterns changed markedly during the flexion stage. Early flexion larvae (ca. 5.0 mm NL) were characterized by the inverted saddle on the caudal peduncle, mass of pigment on the gut that spreads laterally (and may extend to the anterior surface of the gut), embedded melanophores on the perineural sheath in the nape area, and melanophores on the membranous sheath at the distal edge of second first-dorsal-fin and pelvic spines (Fig. 4A). On later flexion larvae (5.7 mm NL), the melanophores forming the inverted saddle on the caudal peduncle migrated dorsad, and pigmentation on the tip of the upper and lower jaw began to form. These larvae were further characterized by the mass of pigment spreading laterally over the gut, melanophores on the anterior surface of the gut, embedded melanophores on the perineural sheath in the nape area and melanophores on the second first-dorsal-fin and pelvic spine sheaths. On the latest flexion larvae, pigmentation on the caudal peduncle was well defined. The pattern consisted of a patch of melanophores on the lateral surface, halfway between the lateral line and the ventral midline and embedded melanophores on the perineural sheath (Fig. 4B). An embedded melanophore on the posterior surface of the optic lobes (or between the front and mid-brain) first appeared at this stage. In some specimens, a few melanophores occurred on the top of the head. The pigment pattern on young postflexion larvae ($\leq$6.8 mm SL) was similar to that of late flexion larvae, but was denser and more extensive (Fig. 4C). Young postflexion larvae were

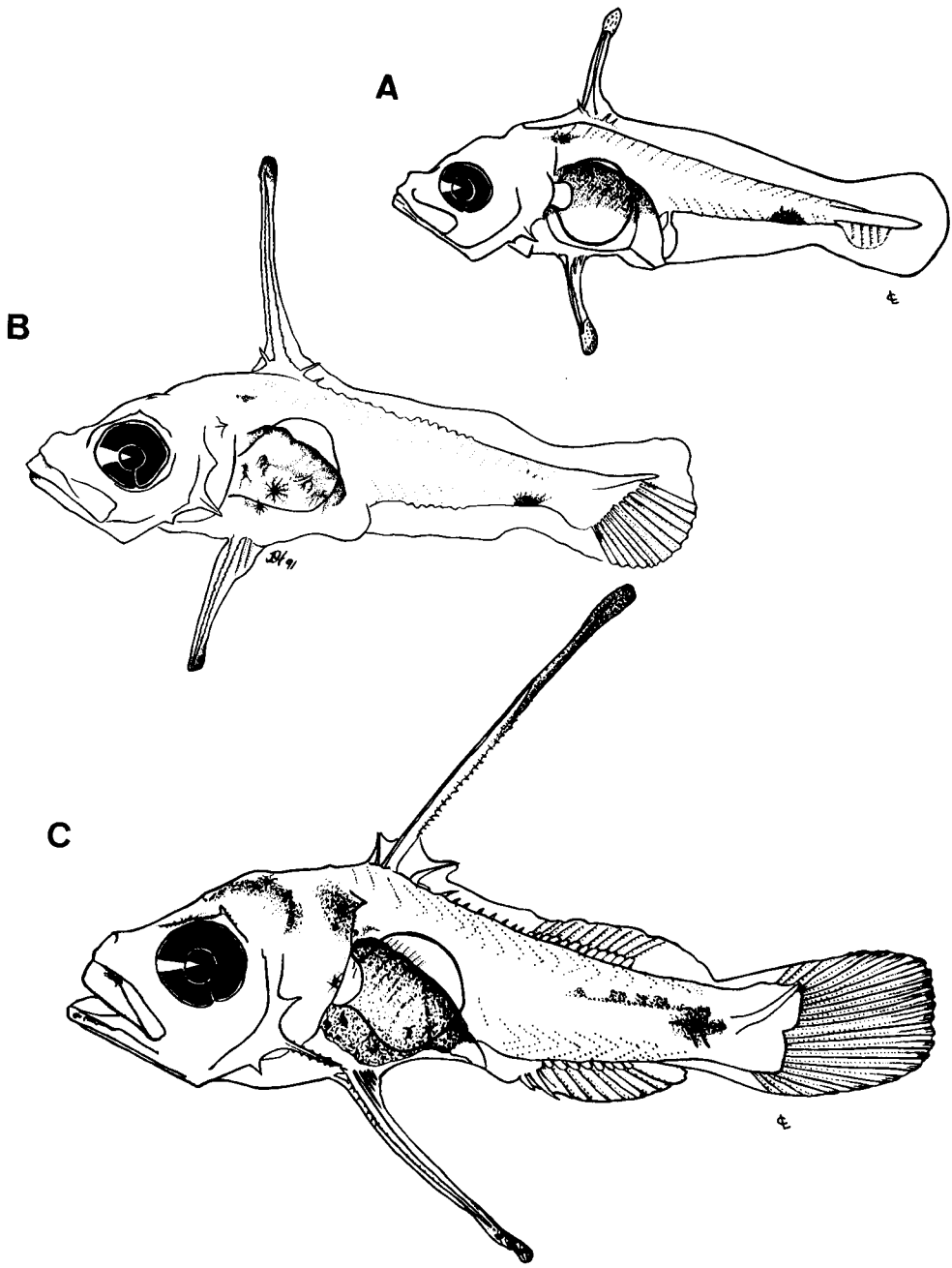

Figure 4. Developmental stages of laboratory-reared *Epinephelus striatus*: A, 4.9 mm NL early flexion larva, 13 days old; B, 6.2 mm NL late flexion larva, 20 days old; C, 6.8 mm SL postflexion larva, 25 days old.

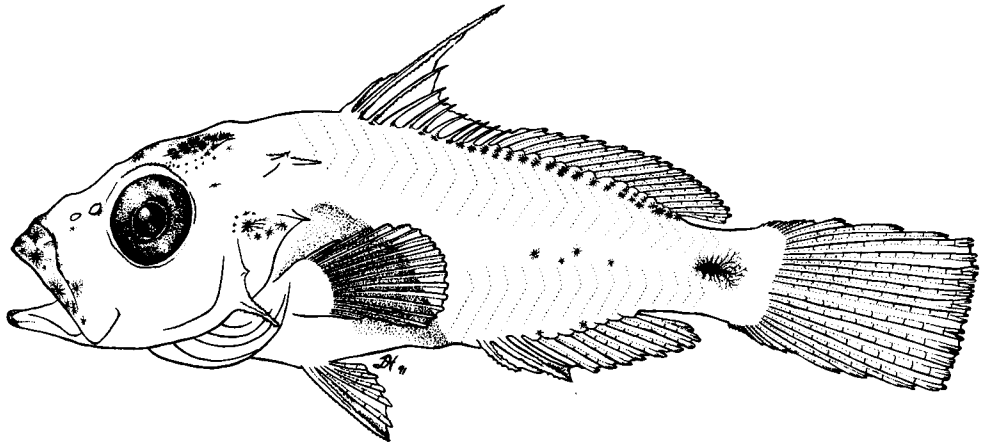

Figure 5. Laboratory-reared postflexion *Epinephelus striatus*, 13.5 mm SL, 40 days old.

characterized by a patch of pigment on the lateral surface of the caudal peduncle, embedded melanophores on the perineural sheath over the caudal vertebrae extending anteriad of the caudal peduncle, dense embedded melanophores on the perineural sheath at the base of the hindbrain (best seen in cleared and stained specimens), a mass of pigment on the surface of the gut including the anterior surface, pigment on the second dorsal and pelvic spine sheaths, embedded melanophores on the nape of the posterior surface of the optic lobes, melanophores on the top of the head, and melanophores on the tips of the lower and upper jaws (Fig. 4C). Larger postflexion larvae (7.4 mm SL) attained additional pigmentation. Melanophores began to appear at the base of the dorsal fin and, in some specimens, on the distal edge of the anal fin spines. Most larvae had a melanophore at the base of the second and third first-dorsal-fin spines whereas the number of melanophores on the bases of other fin elements varied. For example, one specimen (8.3 mm SL) had a melanophore on the 11th through 17th fin elements, while another (9.4 mm SL) had melanophores at the bases of the second, third and eleventh spines and at the base of the first through ninth fin rays. Also, on larger postflexion larvae, pigment became more extensive at the dentary, premaxilla and maxilla. At approximately 8.7 mm SL embedded pigment along the perineural sheath extended the full length of the notochord. This was best seen in cleared and stained material. At approximately 9.2 mm SL a swath of pigment began to form between the eye and gut (hyomandibular/opercle region). At this size only a few punctate melanophores were present, but by 12.7 mm SL the swath was well defined. Additional pigment added at this size consisted of faint stellate melanophores at the base of the anal fin.

The pigment pattern on the largest specimen (13.5 mm SL) was similar to that of smaller postflexion larvae, but more extensive (Fig. 5). Melanophores occurred at the distal edge of the second and third dorsal spines and all along the base of the dorsal fin, with a stellate melanophore approximately at each pterygiophore. Two to three faint stellate melanophores occurred at the anal fin base. The pelvic fin was pigmented, especially at the distal end of the spine. The caudal spot was distinctive, and anterior to this spot along the midline, stellate melanophores began to develop. Melanophores also appeared on the body near the massive patch of gut pigmentation. Heavy external pigment occurred over the forebrain and optic lobes, and embedded melanophores were present in the hindbrain area.

Table 3. Distributional range and spawning season of western Atlantic species of *Epinephelus*. Numbers in parentheses indicate the source (see end of table for sources)

Species	Distribution	Spawning season
E. striatus	Venezuela to Florida, Bermuda, Caribbean and Gulf of Mexico (8). Brazil to North Carolina (1).	Jan–Apr near Jamaica (9). May–Aug near Bermuda (8). Dec–Mar near Cayman Island (10).
E. fulvus	Brazil to Florida, Bermuda, Caribbean and Gulf of Mexico (8). North Carolina (3).	Nov–Jul near Jamaica (9). May–Aug (8).
E. cruentatus	Brazil to Florida, Bermuda, Caribbean and Gulf of Mexico (8). North Carolina (3).	Apr–May (at least) near Jamaica (9).
E. morio	Trinidad to North Carolina, Bermuda, Caribbean and Gulf of Mexico (8). Brazil (1). Massachusetts (7).	Mar–Jul off west coast of Florida (6).
E. guttatus	Venezuela to South Carolina, Bermuda, Caribbean and Gulf of Mexico (8). Brazil to North Carolina (1).	Jan–Apr near Jamaica (9). May–Jul near Bermuda (2).
E. niveatus	Brazil to Massachusetts, Caribbean and Gulf of Mexico (8).	—
E. flavolimbatus	Brazil to Florida, Caribbean and Gulf of Mexico (8). North Carolina (5).	—
E. nigritus	Brazil to Massachusetts, Caribbean and Gulf of Mexico (8).	—
E. mystacinus	Venezuela to Florida, Bermuda (8) Brazil (1).	Ripe female captured in Aug and Nov near Jamaica (9).
E. guaza	Brazil (8).	—
E. drummondhayi	Florida, Bermuda and Gulf of Mexico (8), North Carolina (4).	—
E. adscensionis	Brazil to Massachusetts, Bermuda, Caribbean and Gulf of Mexico (8).	—
E. itajara	Brazil to Florida, Caribbean and Gulf of Mexico (8).	—
E. inermis	Brazil to Florida, Caribbean (8). North Carolina (3).	—
E. *afer*	Brazil to Florida, Bermuda, Caribbean (8).	Ripe female seen in July (8). Ripe fish seen in Dec near Jamaica (9).

1 Böhlke and Chaplin (1968).
2 Burnett-Herkes (1975).
3 Dixon, R. L. National Marine Fisheries Service, NOAA, Southeast Fisheries Center, Beaufort Laboratory, Beaufort, North Carolina 28516, pers. commun. April 1990.
4 Hoese and Moore (1977).
5 Huntsman (1976).
6 Moe (1969).
7 Randall (1983).
8 Smith (1971).
9 Thompson and Munro (1983).
10 Tucker, J.W. and P.G. Bush. Spawning locations and times of Nassau grouper (*Epinephelus striatus*) near Grand Cayman. Unpubl. manuscr. Harbor Branch Oceanographic Institution, Fort Pierce, Florida 34946.

External pigment appeared on the snout just anterior to the eye. The premaxilla and maxilla and anterior portion of the lower jaw were heavily pigmented.

Distinguishing Epinephelus striatus *from Other Epinephelin Serranids.*—*Epinephelus striatus* co-occurs and shares a similar spawning season with numerous other epinephelin serranids (groupers) (Table 3). Larval groupers all share a characteristic appearance—elongated and serrated second dorsal and pelvic spines, serrated

spine at the angle of the preopercle, serrate ridge above the eye, protruding serrate spine on the posttemporal and supracleithral bones, a few melanophores on the surface of the optic lobe, some on the lateral surface of the gut, and a patch of pigment on the caudal peduncle that migrates dorsad during development (Kendall, 1979; 1984; Johnson and Keener, 1984).

It is highly unlikely that preflexion and flexion epinephelin larvae can be positively identified as *E. striatus* until a comparative study of epinephelin larvae is made. Even then caution must be used as pigment patterns of preflexion and flexion groupers, worldwide, appear similar and differences could be influenced by rearing conditions. For example, illustrations of preflexion *E. akaara* show dense pigment on the body dorsal to the gut that is absent in an early flexion larva (Ukawa et al., 1966; Mito et al., 1967). Whether this dense body pigment is characteristic of this grouper or a result of rearing conditions is difficult to discern; however, this same pattern is described for *E. malabaricus* (Manneewong et al., 1986). Illustrations of *E. amblycephalus* show atypical grouper pigment (Tseng and Chan, 1985). There is no patch of pigment at the caudal peduncle. Whether this is an omission by the authors or an accurate description of the larvae cannot be determined. If this is an accurate description, then it is the only larval grouper that has been described that does not have a patch of pigment on the caudal peduncle that migrates dorsad during development (Kendall, 1979; 1984; Johnson and Keener, 1984).

Pigment that is diagnostic for separating groups of epinephelin serranids is the presence or absence of a melanophore on the ventral cleithral junction. This melanophore does not occur on *E. striatus,* but is present on other co-occurring epinephelins—*Mycteroperca* spp., *E. niveatus* and *E. flavolimbatus* (Johnson and Keener, 1984). The melanophore appears to be present on larvae of these species by at least 4.0 mm NL (Johnson and Keener, 1984).

Later preflexion Nassau grouper larvae (4.6–5.0 mm NL) have spinelets (simple, straight, and small) on both the second first-dorsal-fin and pelvic spines that could separate them from all other epinepheline larvae except *E. adscensionis/morio/guaza/drummondhayi/guttatus* (Johnson and Keener, 1984). Caution should be used here because comparative studies are needed to determine if spinelet patterns as described for older larvae (Johnson and Keener, 1984) apply to preflexion and flexion larvae. Regardless of species, spinelets may initiate development as simple, straight, small structures and later develop a more complex morphology.

Length of the second first-dorsal-fin and pelvic spines may be a useful character in separating epinephelin larvae (Johnson and Keener, 1984). For example, larval *E. niveatus* have extremely long second first-dorsal-fin (73% of SL at 5.5 mm SL; 99% of SL at 6.2 mm SL) and pelvic spines (49% of SL at 5.5 mm SL; 40% of SL at 6.2 mm SL) (Presley, 1970) relative to *E. striatus* (Fig. 3). However, as with pigment patterns, comparative studies are needed before spine lengths can be used as diagnostic characters.

Based on meristic characters, *E. striatus* can be separated from all other western Atlantic epinephelin groupers except *E. guaza* (known in the western Atlantic only from Brazil), *E. itajara,* and *E. adscensionis* (Johnson and Keener, 1984). We have shown that the adult complement of dorsal spines and rays can be determined for specimens 6.0–6.8 SL, and anal spines and rays for specimens 5.7–6.0 mm NL by using pterygiophores, but bony stays are not completed until 7.4 mm SL. *Epinephelus striatus* can be separated from *E. itajara,* but not *E. adscensionis,* by spinelet morphology and possibly second first-dorsal-fin spine length (Johnson and Keener, 1984). A 9.2 mm SL *E. itajara* specimen examined

by Johnson and Keener had a dorsal spine length of 88% SL. *Epinephelus striatus* at this size have a dorsal spine length that is 32% SL (Fig. 3). This suggests that *E. itajara* probably have longer spines than *E. striatus* throughout the larval stage.

In conclusion, separation of preflexion and flexion Nassau grouper from all other epinepheline groupers does not appear possible until comparative studies dealing with pigment patterns, second-dorsal-fin pterygiophore patterns, ceratobranchial gill raker counts, and spinelet formation are done and because of similarities in pigment among groupers this may not solve the problem. With postflexion larvae, approximately ≥ 7.4 mm SL it appears to be possible to separate the Nassau grouper from all other groupers, except *E. adscensionis,* on the basis of dorsal and anal fin ray counts, spinelet configuration, second first-dorsal-fin spine length relative to standard length, and capture location.

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